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Study protocol for the Melbourne Infant Study: BCG for Allergy and Infection Reduction (MIS BAIR), a randomised controlled trial to determine the non-specific effects of neonatal BCG vaccination in a low-mortality setting

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ABSTRACT

Introduction BCG vaccination reduces all-cause infant mortality in high-mortality settings by more than can be attributed to protection against tuberculosis. This is proposed to result from non-specific protection against non-vaccine targeted ('off-target') infections. There is also evidence that BCG protects against allergic diseases.

Methods and analysis The Melbourne Infant Study: BCG for Allergy and Infection Reduction is a phase III multicentre, single-blinded, randomised controlled trial. A total of 1438 healthy neonates will be randomised to receive either BCG vaccination or no BCG vaccination in the first 10 days of life. Measures of allergy, eczema, infection and asthma will be obtained from parent-completed questionnaires 3 monthly in the first year and 6 monthly from 1 to 5 years of age, and clinical assessments at 1 and 5 years of age. Biological samples will also be collected for future immunological studies.

Analysis primary outcome The proportion of participants with measures of allergy and infection (atopic sensitisation, eczema, lower respiratory tract infection) at 1 and 5 years of age, and asthma at 5 years of age. Secondary outcomes: (1) the proportion of participants with additional measures of allergy, eczema, asthma and infections; (2) medication use for eczema and asthma; (3) the severity and age of onset of eczema and asthma; (4) the number of episodes of infection; (5) hospitalisations for infections and (6) laboratory measures of immune responses.

Ethics and dissemination This trial has ethical and governance approval from Mercy Health Human Research Ethics Committee (HREC, No. R12-28) and Royal Children's Hospital HREC (No. 33025) with additional governance approval from Barwon Health and St John of God, Geelong, Victoria. Results of this trial will be published in peer-reviewed journals and presented at scientific conferences.

Trial registration number NCT01906853

Strengths and limitations of this study

- The use of well-defined, internationally accepted outcome measures in a large-scale randomised trial.
- Low tuberculosis burden in Australia reduces potential influence of *Mycobacterium tuberculosis* exposure.
- Use of oral food challenge in addition to skin prick testing provides a robust and clinically relevant measure of food allergy.
- Inability to blind parents to infants' randomisation group due to the scar resulting from BCG vaccination.
- Routine scheduled non-live vaccines administered subsequent to randomisation might attenuate the beneficial non-specific effects of BCG.

INTRODUCTION

The BCG vaccine is given to more than 85% of infants worldwide to protect against tuberculosis (TB).[1] In addition to protecting against TB, vaccination with BCG-Denmark reduces all-cause neonatal mortality in a high-mortality setting,[2–4] likely by protecting against non-mycobacterial infections.[4 5]

Observational studies suggest that the beneficial 'non-specific' (heterologous) effects of BCG on the developing immune system may also reduce the prevalence of allergic disease and asthma in children.[6] However, meta-analyses of observational studies have had inconsistent findings.[7–9] The two randomised controlled trials (RCTs) that have investigated the effect of BCG on infant allergic disease both found that BCG vaccination reduced infant eczema (medication for eczema at 18

months of age[10]; clinically diagnosed eczema at 13 months of age[11]) but did not have a statistically significant effect on the prevalence of allergic sensitisation or food allergy at 13 or 18 months of age. However, in these studies, allergic outcomes were determined by parent questionnaire and serum IgE, rather than clinical assessment. Studies assessing the impact of BCG vaccination on asthma have had more consistent findings with two meta-analyses concluding the BCG reduces the risk of asthma by 14%–27%.[7 9]

As a result of a reduction in the prevalence of TB in several high-income and middle-income countries, BCG has been removed from routine vaccination schedules. This might have contributed to the increased prevalence of allergic diseases over the past three decades.[12–16] It is proposed that, by acting as an early life microbial stimulus,[17] BCG prevents allergy by skewing the developing immune system in predisposed individuals away from the T helper (Th) 2 type immune response typically associated with allergy.[6 8]

BCG vaccination induces potent Th1 responses in neonates. In adults, it induces trained immunity in innate immune cells[18 19] and promotes Th1 and Th17 responses to non-mycobacterial pathogens.[20–22]

Two large observational studies provide further evidence that BCG vaccination protects against non-mycobacterial infections, particularly sepsis and respiratory infections.[23 24] However, BCG-mediated protection against these non-vaccine targeted or 'off-target' infections might be limited by subsequent vaccination with non-live vaccines which are proposed to counteract the beneficial non-specific effects of BCG.[4 25 26] Studies in a low-mortality setting, including a recent RCT of neonatal BCG vaccination[27] and an observational study of over 19 000 infants,[28] support this hypothesis with protective effects of BCG against off-target infections most evident in early infancy, before administration of non-live vaccines.[29] Moreover, in the RCT, the protective effect of BCG was only evident in infants of BCG-vaccinated mothers.[27]

Subsequent to a 2004 WHO recommendation, an increasing number of countries have implemented routine neonatal hepatitis B vaccination.[30] Therefore, the potential impact of this vaccine on the beneficial non-specific effects of neonatal BCG vaccination also requires consideration.

Australia, where routine BCG vaccination was halted in the 1980s—has one of the highest rates of infant allergic disease globally.[31–33] It, therefore, represents an ideal setting in which to assess the impact of BCG on allergic disease. The Melbourne Infant Study: BCG for Allergy and Infection Reduction (MIS BAIR) is an RCT to investigate whether neonatal BCG vaccination reduces the prevalence of allergic and infectious disease.

STUDY AIMS

Primary aims

To determine whether neonatal BCG vaccination compared with no BCG vaccination, reduces allergic disease, infection and asthma in infants and children in Australia.

Secondary aims

To evaluate the immunological mechanisms underlying the non-specific effects of BCG by comparing the immune responses in BCG vaccinated to those in BCG-naïve infants.

METHODS AND ANALYSIS

Study design and setting

This is a phase III multicentre, single-blinded, RCT of neonatal BCG vaccination compared with no BCG vaccination. Recruitment commenced in August 2013 and study follow-up will be completed by 2022.

The study population will be healthy neonates 0–10 days of age born at one of the study site hospitals in Victoria, Australia. Recent studies of the burden of allergic diseases in this population have reported high a prevalence of eczema (28%[34]) allergic sensitisation (18%) and clinically significant food allergy (10.4%[35]) at 12 months of age, as well as high rates (22%) of asthma by 4–5 years.[36]

Study sites comprise: the Murdoch Children's Research Institute (MCRI), Melbourne; Royal Children's Hospital (RCH) Melbourne; Mercy Hospital for Women, Heidelberg; Werribee Mercy Hospital, Werribee; University Hospital Geelong, Geelong; and St John of God Geelong Hospital, Geelong.

Patient and public involvement

Patients and public were not involved in the design of this study. The results of this study will be disseminated to study participants via participant newsletter.

Eligibility criteria

The inclusion criteria comprise: healthy neonates up to 10 days of age; birth weight greater than 1500 g; gestational age equal to or greater than 32 weeks, mothers testing HIV negative during pregnancy, English-speaking mother and parent/legal guardian able to complete questionnaires in English and attend study visit. The exclusion criteria comprise: any indication for BCG vaccination in the first year of life as per the Australian national guidelines[37]; known or suspected HIV infection; infant at risk of immunodeficiency; serious underlying illness (including fever) or medical instability; skin infection or other skin condition; need for treatment with hepatitis B immunoglobulin; multiple birth of more than twins; older sibling in the study.

Intervention

Infants will be randomised to receive BCG vaccination or no BCG vaccination in the first 10 days of life. Infants randomised to the BCG vaccination group will be administered a single intradermal injection of 0.05 mL *Mycobacterium bovis* BCG vaccine, Danish Strain 1331 ($1–4\times10^5$ colony forming units) over the left deltoid within 24 hours of randomisation.

Reasons for withdrawal

Reasons for withdrawal from the study will be recorded along with demographic data. These may include:

Table 1 Data and sample collection schedule

Infant age	Antenatal	0–10 d	3 m	6 m	9 m	1 y	1.5 y	2 y	2.5 y	3 y	3.5 y	4 y	4.5 y	5 y
Recruitment and randomisation														
Eligibility check and consent	✓	✓												
Baseline questionnaire	✓	✓												
Birth questionnaire		✓												
Randomisation±vaccination		✓												
Full blood examination		✓*												
Post randomisation														
Perinatal hospital data		✓												
Parent questionnaire			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Clinical eczema assessment						✓								✓
Skin prick test						✓								✓
Oral food challenge						✓†								✓†
Biological sample collection		✓‡		✓§		✓								✓

*Soon after birth.
†If indicated by skin prick test result.
‡7±4 days postrandomisation.
§5–8 months of age.
d, days; m, months; y, year.

protocol violations, indication for participant to receive BCG vaccine within the first year of life[37], serious adverse event (SAE) or other AE or guardian/parent request.

Data and sample collection

Timing of data and sample collection in MIS BAIR is summarised in table 1. Web-based questionnaires will be administered to parents at the time of recruitment, randomisation and when the participant is 3, 6, 9 and 12 months of age as well as 6 monthly up to 5 years of age using the Research Electronic Data Capture (REDCap) platform.[38] Infant perinatal data, including infections, medications and hospital admission, will be obtained from the birth site records. Participants will be invited to optional study visits for biological sample collection 7 days (±4 days) after randomisation and at 6 months (5–8 months) of age. Infants will be invited for clinical assessment and biological sample collection at 1 year (between 11 and 24 months) and 5 years (between 5 and 6 years) of age.

Parent questionnaires

To collect data on potential confounding factors and for stratification prior to randomisation, baseline parent questionnaires will be used to collect data on demographic, environmental, prenatal and birth factors (table 2). When the participants are 3, 6, 9 and 12 months of age, questionnaires will be provided to parents to collect data on diet, medications and potential environmental confounders, and to collect data for primary and secondary outcomes (table 3).

Clinical assessments

Allergic sensitisation: skin prick testing (SPT) to the following panel of allergens will be assessed: food allergens—cow's milk, raw egg, peanut, sesame, cashew, hazelnut, shellfish, walnut (at 5-year visit only); other allergens—*Dermatophagoides pteronyssinus 1* (house dust mite), cat, dog, *Alternaria tenuis* (mould) and rye grass pollen. SPT will be done according to standard guidelines.[39] For

Table 2 Baseline questionnaire data

Family and demographic	Family history of allergic disease (allergy, eczema, hay fever, asthma), maternal BCG vaccination, parent education, parent country of birth, ethnicity.
Household environment	Size, composition, smoking, pets, region (postal code).
Maternal prenatal	Age, weight, height, prior pregnancies, vaccinations, smoking, antibiotics, vitamin D, probiotics other medications or supplements.
Birth	Mode of delivery*, birth site*, plurality*, birth complications, gestational age, weight, sex, antibiotics or other medications during labour.

*Required for stratification prior to randomisation.

Table 3 Three and six monthly questionnaire outcome data

Illnesses	Any episode of illness, infant age, duration of illness, symptoms, medial consultations and hospital admissions, diagnosis and any treatments/medications.
Eczema	Symptoms of eczema, infant age at onset of eczema, distribution of eczema, use of eczema medications, medical consultations and hospital admissions. Includes modified U.K. working party's diagnostic criteria for atopic dermatitis and modified patient-oriented eczema measure (POEM) tool questions.[52 53]
Allergies	Any episodes of food or other allergies, infant age, allergen, symptoms, severity, diagnosis and any treatment.
Asthma	International Study of Asthma and Allergies in Childhood (ISAAC) questions,[54 55] asthma medication usage, symptom severity, acute exacerbations and healthcare utilisation, including hospitalisations.
Diet	Breast milk feeding, formula milk feeding, food introduction, dietary supplements.
Other	Household composition, childcare, pets and other animals, household smoking, drinking water source, vaccinations, other medications or supplements, other diseases/disabilities, BCG complications, overseas travel, non-illness associated hospital admissions.

each food allergen tested, data will also be collected on prior ingestion, exposure, reactions and tolerance (after the SPT weal size is assessed).

Eczema: Severity of eczema will be assessed using Scoring of Atopic Dermatitis (SCORAD).[40]

Oral food challenge (OFC): Participants with a weal diameter ≥1 mm greater than the negative control to selected food allergens during SPT will be invited to have an OFC as detailed in online supplementary figure 1. OFCs will be done at MCRI according to Australasian Society of Clinical Immunology and Allergy guidelines as used by the RCH Allergy Clinic and the HealthNuts study.[41] The different pathways that will occur during an OFC detailed in online supplementary figure 2.

Other: Weight, height, skin, eye and hair colour, BCG vaccination site including scar measurement and photograph. BCG vaccination site assessment will occur after all other assessments are complete.

Biological sample collection

Full blood examination: A capillary blood sample will be collected from participants soon after birth as a preliminary screen for primary T-cell immunodeficiencies. This may be done before or after randomisation.

Peripheral blood: Peripheral blood samples will be collected at 7 days, 7 months, 1 year and 5 years of age. These samples will be used for immediate immune stimulation experiments as well as separation and storage of peripheral blood mononuclear cells and granulocytes, plasma and plasma-depleted cells at −80 °C or in liquid nitrogen, as appropriate. These samples will be retained for future immunological analysis.

Stool: From the day of birth, participant's parents will be requested to collect a stool sample on each day (up to seven samples) and store it in a domestic freezer until collection at the 7-day postrandomisation study visit. Additional stool samples will be collected during the 7-month and 1-year study visits or by parents prior to the 1-year study visit using an in-home stool collection kit provided by the study team.

Outcomes

Primary outcomes

The primary outcomes are the proportions of infants with the following measures of allergic disease and infection at 1 year and 5 years of age:

- Atopic sensitisation (positive SPT) to one or more of a panel of food and aeroallergens.
- Eczema (based on Williams' UK diagnostic criteria).
- Lower respiratory tract infection (LRTI) episode ever (1 year) or hospitalisation ever (5 years).
- Asthma (ISAAC definition) ever and current (5 years of age only).

Secondary outcomes

The secondary outcomes are the following additional measures of allergy and infection:

Allergy

The proportion of infants with clinical food allergy (online supplementary figures 1 and 2); atopic sensitisation with ≥3 mm weal diameter to any allergen; atopic sensitisation to multiple allergens; parent report of food allergy to any food; atopic sensitisation to egg allergen; clinical egg allergy; atopic wheeze.

Eczema

Severity of eczema; age of onset of eczema and proportions of clinically diagnosed eczema and steroid use for eczema.

Infections

Proportion of infants with or rate of the following: any infection; upper respiratory tract infection; LRTI; diarrhoea with vomiting; rash with fever; fever; hospitalisation for infection; hospitalisation for respiratory tract infection.

Asthma

The proportion of infants with current asthma, asthma severity and medication use for asthma.

Sample size and power calculation

This study aims to randomise a total of 1438 participants. This sample size is calculated based on an assumed

minimum 80% retention rate, resulting in an expected minimum 1150 infants with 1-year data available. With a final sample size of 575 in each arm, this study is powered to detect a minimum 35% reduction in atopic sensitisation, 25% reduction in eczema and 25% reduction in LRTI with a power of 80% in the first year of life and a minimum 37% reduction in atopic sensitisation, 26% reduction in eczema, 26% reduction in LRTI and 27% reduction in asthma with a power of 80% at 5 years of age. These differences are based on the previously reported prevalence of atopic sensitisation, eczema, LRTI and asthma in Australian infants at 1 and 5 years of age.[35 36 42]

Recruitment

Recruitment will occur in two stages: (1) early consent will be sought from pregnant women attending antenatal clinics or in the postnatal ward at a study site; (2) additional pregnant women or mothers interested in participating but not being cared for at a study site may also be recruited if they contact the research team antenatally or within 10 days of delivery. Consent to participate in the study will be verbally confirmed after the birth of antenatally recruited infants and prior to randomisation.

Randomisation

Recruited neonates will only be randomised after confirmation that they still fulfil inclusion criteria and do not meet any exclusion criteria. Randomisation to BCG vaccination or no BCG vaccination will be done in a 1:1 ratio using the REDCap randomisation function.[38] The randomisation schedule will be established by a statistician external to the study using random permuted blocks with a minimum of three different block sizes. Randomisation will be stratified by: (1) site (hospital); (2) method of delivery (Caesarean vs non-Caesarean) and (3) plurality of birth (twins vs singletons). Twins will be assigned to the same intervention arm.

Blinding

As BCG vaccination results in the formation of a scar at the vaccination site in 93%–99% of infants,[43–46] blinding with the use of a placebo is not possible. For blinding of clinical assessments, prior to commencement of the 1-year and 5-year study visits, each participant's left upper arm will be covered with a bandage by the parent or a member of the study team not involved with the assessment to hide the potential scar site. Clinical assessments will be done by a member of the study team or clinical staff member who was not involved in the randomisation of the participant.

Statistical analysis

Statistical analysis of primary and secondary outcomes will be overseen by the trial statistician. Primary analysis will be by intention to treat, including all randomised participants where outcome data are available. Data will be collected according to Consolidated Standards of Reporting Trials guidelines for reporting randomised trials.[47]

Outcomes

Primary outcomes

Comparison between BCG-vaccinated and BCG-naïve infants will be estimated using binary regression adjusted for the stratification factors used during randomisation. Results will be presented as risk difference with 95% CIs.

Secondary outcomes

Comparison between the BCG-vaccinated and BCG-naïve infants will be estimated using binary linear or Poisson regression adjusted for the stratification factors used during randomisation. Results will be presented as risk difference with 95% CI.

Missing data

If the proportion of missing data is less than 5%, the primary analysis will be a complete-case analysis. Otherwise, the frequency and patterns of missing data will be examined and, if appropriate, multiple imputation models will be conducted for the outcome variables. Fifty completed data sets will be imputed by chained equations including all the children initially randomised. The primary outcome, strata variable (mode of birth: vaginal/caesarean) and the variables predictive of missingness and allergy, eczema, infection or asthma will be included in the imputation model.

Subgroup analysis

Prior to any subgroup analysis, adjusted models including the stratification factors used in randomisation, the randomisation assignment and the subgroup variable as covariates will be used to estimate the interaction between the intervention and the subgroup variable. Where these models provide evidence that the intervention varies between subgroups, specific subgroup estimates and CIs will be presented obtained from the adjusted model. The subgroups are presence or absence of BCG scar; timing of BCG administration; maternal BCG vaccination; sex; mode of delivery; season of birth; timing of hepatitis B vaccination. For allergy, eczema and asthma outcomes, an additional subgroup variable, family history of allergic disease or asthma, will be assessed.

Data monitoring and auditing

The independent data safety and monitoring committee (DSMC), consisting of an independent statistician, neonatologist and paediatric infectious diseases consultant, will meet to review data and participant safety 6 monthly.

Risks

The potential risks of participation in this study may be related to: (1) adverse reactions to BCG vaccination: subcutaneous abscess, exaggerated local reaction, lymphadenitis, keloid scaring, osteitis and disseminated infection.[48] In Australia, adverse reactions to BCG vaccine occur in 15.3 out of 10 000 doses[49]; (2) blood collection: discomfort, bruising and rarely minor infection or blood clots (3) clinical assessments of allergy: anaphylactic reactions may occur during OFC, during SPT (rare) and as

a late reaction to OFC (from further allergen exposures during the subsequent week). The protocols and workflows for SPT and OFC mitigate the risk of anaphylactic reactions and their safety has been demonstrated in previous clinical trials (online supplementary figures 1 and 2).[41] All AE will be recorded, and SAEs reported to the study site Human Research Ethics Committee and the DSMC.

Outlook and significance

The findings of MIS BAIR will provide evidence as to whether neonatal BCG vaccination, a low-cost, readily available intervention, reduces the prevalence of allergies and infections in the first one and 5 years of life, and asthma in the first 5 years of life. An immune priming benefit of neonatal BCG against these diseases would have considerable public health implications and thus inform guidelines for BCG vaccine policies worldwide.

Limitations

The potential limitations of MIS BAIR include the inability to blind the parent(s)/guardian(s) to the infant's randomisation assignment. This may lead to bias or lower compliance if parent(s)/guardian(s) are disappointed with the randomisation assignment. However, blinding will be done for the 13-month study clinical assessments from which several of the primary and secondary outcomes measures of allergy and eczema are obtained. We expect that there will be greater recruitment of participants with a family history of allergic disease, which may limit the generalisability of our findings. We will, therefore, collect data in relation to family history to enable us to consider for this in later analyses. BCG was followed by multiple administrations of non-live vaccines, and this might attenuate the beneficial non-specific effects of BCG.[50 51]

ETHICS AND DISSEMINATION

A record of consents and refusals will be maintained on the study REDCap database. Parent(s)/guardian(s) of participants will be informed of their option to withdraw from the study at any time. An electronic or hard copy parent/guardian information and consent form will be completed a parent/guardian as part of the consent process. Results of this trial will be published in peer-reviewed journals and presented at scientific conferences.

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Contributors NC is the lead investigator and responsible for study conception, design and funding acquisition. KG developed the trial database. KG, NC, BF, SD, LC and CZ developed the ethics application and all other authors provided critical evaluation and revision. DC, KG, PV, CM, VA and NC developed the recruitment methods. KG, BF, VA, AL-P, KJA and PV developed and SD, MS and NC contributed to the design of the questionnaires. KG and KJA developed and AL-P, NC and PV contributed to the clinical assessment methods. CZ, NC, BF, SG and KF developed and KG, NLM, RR-B and PV contributed to biological sample collection and processing methods. SD developed the statistical analysis plan. NC, KG, KJA, FS, VA, NLM and MS contributed to and AL-P, KF, RR-B, PV and DC provided critical evaluation and revision. NLM drafted the manuscript, coordinated manuscript preparation and revision. PZ contributed to an early draft of the manuscript. All authors provided critical evaluation and revision of the manuscript, and have approved the final version of this manuscript.

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Competing interests None declared.

Patient consent for publication Not required.

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